EP1.89/2:600/51-85-007

United States
Environmental Protection
Agency

Health Effects
Research Laboratory
Research Triangle Park NC 27711

Research and Development

EPA 600 S1-85 007 Aug. 1985

EPA

Project Summary

ORIGINAL

Teratogenic Effects of Microwave Radiation

Mary Ellen O'Connor and Robert Strattan

Pregnant CF-1 mice were exposed to 2450-MHz CW microwave irradiation at power densities of 0, 10, or 30 mW/cm^2 for 6 hours daily from gestational day 1 through day 18. All exposures occurred in an anechoic chamber maintained at 50% relative humidity with air temperature of 22°C. A group of pregnant, cage-control colony mice was maintained for each of the three exposure groups. Teratogenic examinations were performed on day 18 following Caesarean section of the maternal subject. Every third fetus was stained with Alazarin red and examined for skeletal abnormalities. No significant differences between the groups were observed for fetal body mass, fetal brain mass, resorption, live fetuses, gross abnormalities, or skeletal abnormalities.

Subsequent experiments were performed on pregnant CF-1 mice at 0, or 30 mW/cm^2 at ambient temperatures of 20°C or 25°C. Embryopathic effects at these levels of irradiation were highly dependent upon the ambient temperature in the chamber during the 6 hours of daily exposure.

Pregnant albino guinea pigs were exposed on days 18 through 25 of gestation for 60 minutes to 2450-MHz CW microwave radiation at a power density of 47 mW/cm^2. Caesarean sections followed by teratogenic examinations were performed on day 60 of gestation. Statistical analysis indicated that the microwave exposed litters had lower average brain mass and female conceptuses had a lower average body mass than sham-exposed controls.

The full report was submitted in fulfillment of contract 68-02-3453 under the sponsorship of the U.S. Environmental Protection Agency.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

At the initiation of this investigation, the data on the teratogenic effects of microwave radiation had been obtained nearly exclusively from a single acute exposure to 2450-MHz CW radiation at power densities well above 10 mW cm^2. Gross teratogenic effects following such microwave exposure did not appear. However, one observation that was reported consistently was reduced average fetal body mass in microwave exposed fetuses. Such a functional deviation as reduced fetal body mass, although mild in comparison to fetal death or the appearance of gross structural abnormalities, is considered as an indicator of less healthy offspring.

The second general observation regarding the teratogenic effects of exposure to microwave radiation was that even these relatively mild functional deviations were observed only at high levels of exposure that clearly elevated core temperature of the maternal organism. Both the rate of temperature increase and the temporal duration over which the maternal organism must endure this high temperature were reported to be important variables in the production of teratogenic effects. The maternal temperature required to induce effects was reported to be 40 C-42 C which is just below the lethal threshold for the maternal subjects.

BEST COPY AVAILABLE

Exposure conditions employed in the laboratory do not resemble exposure conditions found in the general environment. However, the conditions in the general environment are of the highest concern with regard to risk of exposure of the general population. In the general population, exposures are for long periods of time (from days to a lifetime) to what would be considered extremely low levels in comparison to the intensities used in the laboratory.

The purpose of the studies was to obtain information with respect to relatively long-term, low-level exposure to 2450-MHz CW. The CF-1 laboratory mouse was chosen as the subject for the study to facilitate generalization and comparison with earlier reports. This same rationale guided the selection of 2450-MHz CW plane wave radiation. The full report presents results from exposure of mice at 0, 10.0, and 30.0 mW cm^2 as well as results from several additional studies performed at 0 and 30 mW cm^2 at ambient temperatures of both 20 C and 25 C.

An additional study investigated the teratogenic potential of microwave radiation in a larger mammal, the guinea pig. The guinea pig was chosen because of size and because teratogenic effects resulting from hyperthermia induced by non-microwave sources has been reported for the guinea pig. These hyperthermia studies provided a basis for the design of the microwave investigations on the guinea pig. The post-exposure temperature of the maternal guinea pig subject associated with the observation of deleterious effects from hyperthermia was 41 C. The initial microwave study attempted with the guinea pig resulted in average maternal core temperatures just below this 41 C level and did not produce any observable defects in the conceptuses. Since the average maternal temperature did not actually reach the same level as was reported to induce effects from non-microwave induced hyperthermia, the study was repeated at a higher power density.

To summarize, an investigation was conducted to further delineate whether low-level, long-term exposures of CF-1 mice and guinea pigs to 2450-MHz CW microwave radiation would be embryopathic or teratogenic.

Experimental Procedures

Four separate studies were conducted. All experiments were performed at a frequency of 2450-MHz CW microwave radiation in an anechoic chamber with controlled temperature and humidity.

In the first study the exposure conditions consisted of power densities of 0, 10, or 30 mW cm^2 with concurrent cage control subjects maintained in the colony during the exposure period for each group. The subjects were sperm positive CF-1 mice bred in the laboratory at the University of Tulsa. Each morning the females were checked for sperm plugs, and the presence of a sperm plug was counted as day 1 of the gestation period. At this time the female was assigned randomly to either the current exposure or cage control condition.

After the identification of a sperm plug and on each of the subsequent 17 days each female was removed from the group cage, assessed for body mass, placed in a plastic container transported to the anechoic chamber located in an adjoining room, and placed in one of the 18 available positions on the styrofoam platform in the chamber. The position of each mouse in the anechoic chamber was rotated daily so that by the end of the 18-day exposure period, each mouse had occupied each available position for one day of exposure. The subjects were exposed for 18 consecutive days for 6 hours beginning at 0900h and ending at 1500h. Male, nonbreeder mice of the same strain were used for fillers if 18 sperm positive females were not available on a given day. Each of the exposure conditions was continued until 25 gravid mice had been confirmed by Caesarean section. The ambient temperature in the chamber was maintained at 22 C and the relative humidity was 62% in this experiment.

Following exposure on day 18, the maternal subjects were euthanized by cervical dislocation. Caesarean sections were performed by making longitudinal abdominal incisions that exposed the uterine horns. The number and placement of resorptions and visible fetuses was noted and the conceptuses were removed. The fetuses were sexed, viability was noted, and they were examined for the presence of any gross structural abnormality, including cleft palate. Body mass was determined on a Mettler PC440 top-loading balance. The fetal brain was removed, placed in a weigh boat and weighed. Every third fetus was placed in KOH for later staining with Alizarin red to observe skeletal structure. After staining, the fetuses were stored in glycerin and examined under a light microscope for the number of sternal ossifications and number of ribs. At this time any other observable skeletal abnormality also was noted.

Experiment 2 was conducted using CF-1 mice and the same breeding and exposure procedures as Experiment 1, with the exception that the anechoic chamber was maintained at either 20 C or 25 C and only two power density levels were compared: 0 and 30 mW cm^2. The conditions were run sequentially and colony cage control animals were maintained for each of the exposure groups. Caesarean sections, fetal observations, and staining procedures were performed as for Experiment 1.

For the third experiment, the subjects, breeding, and exposure procedures were identical to those for Experiments 1 and 2, with the exception that the animals were exposed for only 100 minutes to either 0 or 30 mW cm^2 with the temperature of the anechoic chamber again maintained at 22 C. Colony cage control animals were maintained for both exposure periods. Caesarean sections, fetal observations and staining procedures were the same as outlined for the earlier experiments.

The subjects for the guinea pig study were Hartley (HA)BR guinea pigs obtained from Charles Rivers Laboratories. Estrus for the females was determined by examining for vaginal opening each day until the membrane covering had receded. The female then was placed with the male and checked daily for the presence of a sperm plug. The day a plug was observed was scored as day 1 of gestation. At this time the animal was assigned randomly to either the colony cage control group or the current (0 or 47 mW cm^2) exposure condition.

Once a sperm plug had been detected the females were left in their home cages and handled only for routine cage maintenance through day 17 of gestation. The exposures occurred on days 18 through 25 of gestation. The animals were assessed for body mass, placed in the plastic cylindrical exposure containers, transported to the anechoic chamber located in the adjoining room, and exposed for 60 minutes. Four animals were exposed at one time and nonbreeding males were used as fillers when necessary. The temperature in the anechoic chamber was maintained at 22 C and the relative humidity was set at 50%. The animals were placed 46 inches below the horn antenna and the

BEST COPY AVAILABLE

power density at this location later was determined to be 47 mW cm^2. This location was chosen because it resulted in maternal post-exposure core temperatures of 41 C after 60 minutes of exposure. The 0 or the 47 mW cm^2 exposures occurred during the same two-hour period each day (1500h to 1700h) and the two conditions were rotated so that on one day the 0 mW cm^2 group was exposed during the first hour and on the subsequent day the 47 mW cm^2 group was exposed during the first hour. Both pre- and post-exposure core temperatures were determined with a Bailey BAT8C thermometer using a RET-3 rectal probe. From day 26 until day 60 of gestation, the animals remained in their home cages and were handled only for routine cage maintenance.

On day 60 the females from all three groups were sacrificed by inhalation of chloroform fumes. Caesarean sections were performed by making a ventral longitudinal incision that exposed the left and right horns of the uterus. The position of resorptions and viable conceptuses was noted prior to their removal from the uterus. All fetuses were sexed, assessed for body mass, and crown-to-rump length was measured. The fetuses were also examined for any observable structural abnormality, including cleft palate. The fetal brain was then removed, placed in a weigh boat, and assessed for mass.

Results and Discussion

All data from this investigation were analyzed using analysis of variance available in statistical packages from SPSS and BMD. All analyses, including those pertaining to microwave exposure parameters, were performed only on data obtained for females that proved to be gravid following the Caesarean section. For each experiment, the initial analysis was a one-way analysis of variance for each endpoint. Duncan's multiple range tests were used to determine differences between individual groups.

As shown in Table 1, a total of 114 gravid dams were used in the analyses for the CF-1 mouse exposed at 0, 10, or 30 mW cm^2. After determining that none of the cage control groups differed significantly for any of the variables of interest, a random sample of 27 cage control litters was used in the one-way analysis of variance.

No differences in average litter fetal body mass, average litter fetal brain mass, number of resorptions, or number of abnormal fetuses were observed. When the ambient temperature was raised to 25 C the fetal body mass of the microwave exposed litters was reduced and the number of abnormal fetuses was increased.

Table 2 presents the data from the guinea pig exposures and indicates that only the femal offspring exhibited significant differences in body mass. When individual offspring were analyzed as separate data points both fetal body mass and fetal brain mass differed significantly, with the microwave exposed group being the smallest in both cases.

Conclusions and Recommendations

The studies did not result in observations of embryopathic or teratogenic effects in the CF-1 mouse exposed to 0, 10, or 30 mW cm^2 microwave radiation (2450 MHz) 6 hours daily throughout 18 days of the gestation period. During the studies the temperature in the anechoic chamber was maintained at 22 C which is a common temperature at which microwave investigations have been reported. This relatively low ambient temperature allows the subject to effectively dissipate any increase in core

Table 1. *Means and Standard Errors for CF-1 Mice Exposed to 2450-MHz CW Microwave Radiation for 6 Hours Daily Throughout the Gestational Period*

Ambient Temperature	Treatment	Litters	Fetal Body Mass	Fetal Brain Mass	Number Resorbed	Number Abnormal
22 C	Colony Control	27	1.07 (.01)	0.064 (.002)	1.07 (.23)	0.30 (0.12)
	0 mW cm^2	26	1.03 (.03)	0.062 (.002)	1.35 (.27)	0.62 (.25)
	10 mW cm^2	25	1.04 (.02)	0.063 (.001)	0.80 (.22)	0.36 (.13)
	30 mW cm^2	36	1.05 (.02)	0.065 (.001)	0.75 (.21)	0.50 (.16)
25 C	Colony Control	28	1.12 (.03)[a]	.063 (.002)	1.00 (.22)	0.14 (.07)[a]
	0 mW cm^2	23	1.07 (.03)[b]	.064 (.001)[a]	1.57 (.39)	0.17 (.10)
	30 mW cm^2	19	0.93 (.02)[a,b]	.059 (.002)[a]	1.53 (.42)	0.53 (.21)[a]
20 C	Colony Control	16	1.05 (.08)	.067 (.005)[a]	1.94 (.61)	0.25 (.14)
	0 mW cm^2	13	1.07 (.04)	.134 (.046)[a,b]	0.77 (.32)	0.54 (.29)
	30 mW cm^2	25	1.08 (.03)	.066 (.002)[b]	1.52 (.27)	0.44 (.12)

For each experiment, cells within a column sharing a subscript are significantly different from one another.

Table 2. *Means and Standard Errors for Guinea Pigs Exposed to 2450-MHz CW Microwave Radiation*

	Cage Control	Sham Exposed	Microwave Exposed	Totals
Number of Litters	7	8	10	25
Number of Live Fetuses	2.86 (0.46)	3.13 (0.29)	2.50 (0.31)	2.80 (0.20)
Fetal Body Mass	64.74 (4.15)[a]	73.19 (1.25)[a]	67.36 (1.91)	68.49 (1.54)
Fetal Brain Mass	2.40 (0.07)	2.52 (0.02)	2.21 (0.09)	2.36 (0.05)
Female Body Mass	66.15 (2.51)[a]	75.54 (1.94)[a,b]	66.82 (2.14)[b]	69.39 (1.55)

Within a row, cells with the same subscripts are significantly different from one another.

BEST COPY AVAILABLE

temperature that may result from microwave induced heating during exposure. In the present investigation, the sham animals were in the anechoic chamber for 6 hours daily for 18 days rather than a few minutes or hours on a single day and such a condition could conceivably induce mild cold stress in pregnant mice.

In the second series of studies, power densities of 0 and 30 mW/cm^2 were employed but at ambient temperatures in the anechoic chamber of 20°C and 25°C. The ambient temperature and its interaction with the microwave power level was the key to the decreases in fetal body mass and fetal brain mass. The results support an essentially thermal model for accounting for embryopathic and teratogenic effects following microwave exposure.

In the study on guinea pigs, the microwave-exposed female fetuses had a lower average fetal body mass than controls. Also, overall fetal body mass as well as fetal brain mass differed significantly across groups if individual fetal data was used as the endpoint for analysis rather than the litter mean. No statistically significant differences in the incidence of abnormalities were observed, but all of the abnormal fetuses observed in the entire study were in the microwave exposure group.

The results of the present investigation could be presented as differences in fetal body mass between the microwave and sham exposure groups if the statistical analysis excluded the colony or cage control subjects. Such an analysis would show a significant reduction in fetal body mass for our sham subjects, which without the colony control data might be interpreted as an increase in fetal body mass as a result of microwave exposure. This type of analysis would disregard the fact that the microwave exposed subjects had average fetal body masses that were nearly identical to what was observed in the cage control colony animals. Data that does not contain some reference to colony cage control baseline information is of limited value and must be used with caution in the assessment of conditions that might present a health risk to the general population.

Mary Ellen O'Connor and Robert Strattan are with University of Tulsa, Tulsa, OK 74104.

***Ezra Berman** is the EPA Project Officer (see below).*

The complete report, entitled "Teratogenic Effects of Microwave Radiation," (Order No. PB 85-207 462/AS; Cost: $8.50, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

The EPA Project Officer can be contacted at:

Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

BULK RATE
POSTAGE & FEES PAID
EPA
PERMIT No G-35

Official Business
Penalty for Private Use $300

EPA/600/S1-85/007

BEST COPY AVAILABLE

4

☆ U.S. GOVERNMENT PRINTING OFFICE:1985—559-016/27119